Cultivation of *Agaricus bitorquis* mushroom as an strategy for the Integrated Pest Management of the myceliophagous mite *Microdispus lambi*

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Abstract

BACKGROUND: The phorid fly *Megaselia halterata* Winnertz (Diptera: Phoridae) is the principal vector of *Microdispus lambi* (Acari: Pygmephoroidea) in Spanish *Agaricus bisporus* Lange (Imbach) mushroom farms. This myceliophagous mite does not appear to be a pest in *Agaricus bitorquis* (Quél.) Sacc mushroom crops. This study explores the role of phorid flies as vectors of *Microdispus lambi* in *Agaricus bitorquis* mushroom crops.

RESULTS: The incidence of *M. lambi* in *A. bitorquis* growing substrates did not reach appreciable levels at any point during the growing cycle. The presence of phorid flies in

A. bitorquis farms was normally higher than that in the case of *Agaricus bisporus* Lange (Imbach) species. The percentage of phorid vectors did not statistically differ between both *Agaricus* crops during infection periods. However, by the end of the crop, this percentage had increased only in *A. bisporus* crops, coinciding with a high incidence of mites in the substrate of this mushroom species; *Megaselia halterata* emerging from the mushroom substrate of *A. bitorquis* summer crops did not carry mites as they were absent from compost and casing.

CONCLUSION*: M. halterata* is a pest in Spanish *A. bitorquis* mushroom crops, meanwhile *M. lambi*, its phorectic mite, has shown not to be a pest of this species mushroom farms during the spring-summer growing season. *A. bitorquis* crops could potentially be used as an IPM measure to decrease the incidence and prevent the propagation of the myceliophagous mite *M. lambi* in *A. bisporus* mushroom growing farms.

Key words: *Agaricus bisporus*, mites, phoresis, diptera, *Megaselia halterata*, IPM

1 INTRODUCTION

Mushrooms are considered as a good source of proteins, vitamins, fats, carbohydrates, amino acids and minerals, as well as possessing important medicinal properties[1]. In recent years, the summer white button mushroom *Agaricus bitorquis* has attracted attention as a functional food, e.g. selenium-fortified food[2], and as a source of new drugs, e.g. antitumor polysaccharides, or for its more general antimicrobial activities[3-7].

Despite slight phylogenetic differences, the biology of *Agaricus bitorquis* is little different from that of *Agaricus bisporus* (Lange) Imbach[8-11]. Breeding studies, and even protoplast fusion techniques, have been used to develop interspecies fusants of *A. bitorquis* and *A. bisporus*, searching for strains with high yields and resistance to diseases and fungicides[12-14]. Both mushroom species require almost the same cultivation practices, although *A. bitorquis* prefers higher temperatures and CO_2 levels. *A. bitorquis* is grown in a mesophilic temperature range of 20 to 30 °C, making it a very important mushroom especially for tropical countries[13, 15-17]. In Spain, its growing temperatures are considerably below those mentioned in the literature[18-19], and the natural infection of *A. bitorquis* crops by pathogens such as *Lecanicillium fungicola* var. *fungicola* (Preuss) has been described[20]. Another important difference is that the individual growing stages within the *A. bitorquis* crop cycle are longer than those described for *A. bisporus*, with weekly yields (known as flushes) possible up to the 10th week of cropping[13].

The myceliophagous mite *Microdispus lambi* (Krczal) (Acari: Pygmephoroidea) was detected for the first time in Spain in the summer of 1996, when it caused substantial economic losses[21]. A study of some Spanish mushroom pests points to the phorid fly *Megaselia halterata* Winnertz (Diptera: Phoridae), the main mushroom fly in Spanish mushroom farms[22-23], as the principal vector of *M. lambi* in Spanish mushroom farms. This is because of the high number of phorid flies, which are more abundant than sciarid flies (*Lycoriella auripila* Winnertz (Dipera: Sciaridae)) in Spanish farms[22-23], and due to the high percentage of phorids that carry mites and the number of *M. lambi* that each phorid fly can carry[25].

Megaselia halterata is one of the most serious arthropod pest problems affecting the cultivation of mushroom throughout the world[26-30]. The preference of phorids for different cultivation materials[27-28], different species of mushrooms[31] or, even, different isolates of one particular mushroom species[32] has been studied, and it has been found that they probably support the development of *M. halterata* in different ways.

The control of the myceliophagous mite pest is based on the control of phorid flies, and is usually based on strict hygiene practices in the growing facilities and the application of pesticides[23]. However, the appearance of pesticide resistance problems in flies, the presence of residues in carpophores[24], and the reduction in the number of permitted active substances have led to the use of biological, biotechnological and cultural and physical measures, rather than chemical methods, in an attempt to promote the Integrated Pest Management (IPM) in mushroom crops[33-34].

The aim of this paper is to know if *M. lambi* and *M. halterata* are pests of *A. bitorquis* mushroom farms, and to find out more about the role of phorids as vectors of the myceliophagous mites in this species crops, in order to establish control measures of the pests.

2 MATERIALS AND METHODS

The study was carried out over two summers and one spring periods on six *A. bitorquis* and twelve *A. bisporus* growing farms in Castilla-La Mancha (Spain), with two crop cycles of *A. bitorquis* and four cycles of *A. bisporus* mushroom per period. Each crop was located in a growing room (35*2.5ç*2 m) with a door for access at the front and a

ventilation hole at the rear. Each crop was entirely grown in a single room and completed within 70 days (*A. bisporus*) or 85 days (*A. bitorquis*).

2.1 Incidence of *Microdispus lambi* in mushroom farms

A sampling calendar was established for each of the 6 *A. bitorquis* growing crops studied. Samples were collected at five time points: after incubation (approx. day 20), after the primordia had formed in the upper surface of the growing unit (induction, day 30 approx.), and after harvesting the first flush (F1, day 45 approx.), third flush (F3; day 65 approx.) and fifth flush (F5; day 85 approx.). The same five sampling time points were established for each of the 12 *A. bisporus* crops, but the days after harvesting periods were slightly modified (after F1, day 41; after F3, day 56; after F5, day 70). A total of 30 samples were taken from each crop cycle (six samples per sampling day). To extract the mites, each sample was submitted to an extraction process (20 g) using Berlese-Tullgren funnels. The mites were collected in an ethanol-glycerine-water solution (6+1+3 by volume) and placed in Petri dishes, where they were identified and counted[35]. The parameter defined for the study was the number of *M. lambi* per 120 g of sample.

2.2 Incidence of the mushroom phorid *Megaselia halterata* in the farms

Three double sided (20 * 14 cm) sticky yellow plates (Aragro S.A., Spain) were used to trap the adult flies in each farm. The sticky plates were removed weekly. Eight growing stages were established: incubation, casing, induction and the first (F1), second (F2), third (F3), fourth (F4) and fifth flushes (F5). Trapped diptera were identified by

stereoscopic microscope and counted. The parameter defined for the study was the total number of adult phorid flies trapped per day, for each time point and each farm.

2.3 Study of the phoretic role of *M. halterata* as vector of *M. lambi*

For each farm, a black light lamp (60 cm, Philips TLD 18w/08, Holland), equipped with a plastic sheet treated with a contact insecticide, was installed under the ventilation hole in order to collect the flies. Each farm was visited weekly. On each sampling day a maximum of 48 flies was randomly collected in well-plates (IWAKI Glass, Japan) and taken to the laboratory, where flies were identified by binocular microscope (Nikon SMZ-2T, Japan) and mites that were phoretic on them were also identified and counted. The parameters defined for the study were the percentage of phorids carrying *M. lambi* mites, and the average load, defined as the number of *M. lambi* mites transported by each carrier phorid.

Statistical analyses

The study consisted in a full factorial experimental design with three factors (species of mushroom, season and growing stage) to evaluate their effects on different interest variables. A GLM[36] was developed for each of the variables studied: (i) the presence of the myceliohagous mite *Microdispus lambi* in the growing substrates, (ii) the incidence of phorid *M. halterata* in the mushroom farms, (iii) the percentage of phorid vectors and (iv) the load that the phorids carried, evaluating in each of them the effects of the factors "species" (two levels: *A. bisporus* and *A. bitorquis*), "season" (three levels: summer1, spring and summer2) and "stage" (five-eight levels: incubation, casing, induction, F1,

F2, F3, F4 and F5) as well as their interactions. In the case of *M. lambi*, a total of 90 observations were evaluated for each variable – as a consequence of our full factorial experiment design, consisting of 3 seasonal periods and 5 growth stages, with 4 replicates for *A. bisporus* and 2 replicates for *A. bitorquis* crops. In the case of *M. halterata* and its phoretic parameters, the number of observations increased to 144, resulted of 3 seasonal periods and 8 growth stages, with 4 replicates for *A. bisporus* and 2 replicates for *A. bitorquis* crop. To test whether continuous variables fitted a normal distribution, data was examined using a normal probability plot, standardized skewness and kurtosis, and the Kolmogorov–Smirnov test. A natural logarithmic transformation was used to account some of the observed heterogeneity of variance in the raw data concerning the presence of mites and phorids. An SQRT transformation was used to account some of the observed heterogeneity of variance in the raw data of the percentage of phorids as vectors and load. The effect of the *Agaricus* species and each particular season and growing stage on the variables was tested using indicator variables (or dummy variables) in a multiple regression analysis[37]. These indicator variables (predictor variables) were the different species (k-1 indicator or dummy variables, k =2 levels of species), the season (k-1 indicator or dummy variables, k =3 levels of seasons) and the growing stages (k-1 indicator or dummy variables, k =5 levels of growing stages for the presence of mites, and k=8 of the growing stages for the incidence of phorids and their phoretic parameters), and the interaction of all of them. The general linear statistic test (F-test)[38] was used to test hypotheses about regression coefficients. All the

statistical analyses were performed using the Statgraphics Centurion XV program (Statistical Graphics Corp., Princeton, NJ).

3 RESULTS

3.1 Incidence of *Microdispus lambi* on the farms

Extremely few myceliophagous mites were collected from the *A. bitorquis* crops regardless of the season or stage of the crop cycle (total number of mites captured per farm: 2-100 mites on *A. bitorquis* crops vs 2,915-6,210 mites on *A. bisporus* farms). The GLM developed to check the effect of the three factors ("species" "season" and "stage") as well as their interactions on the studied variables showed "species" and "stage" factors and the interaction between them as being statistically significant ($p<0.001$, F-test) for the variable "presence of mites" in the growing substrates, meanwhile there was no significance for the "season" factor nor its interaction with the remaining factors ($p> 0.05$, F-test) (Table 1).

The multiple regression analysis showed the stage "third flush" as being statistically significant ($p<0.0001$, F-test), explained by the slightly increasing in the level of mites in both *Agaricus* species crops. Of note was the observation that "first flush", "third flush" again and "fifth flush" stages appeared as statistically significant factors but only in the case of *A. bisporus* mushroom crops, clearly increasing the incidence of mites in the growing substrates of this mushroom species (Table 2). In other words, both mushroom species crops showed approximately the same level of infestation by mites until the beginning of the harvesting period. However, the high

increase of the incidence of mites in growing substrates during the last flushes was only detected for *A. bisporus* crops (Figure 1a).

[Table 1]

3.2 Incidence of mushroom phorid fly *Megaselia halterata* on the farms

The average number of adult flies captured per trap and day in *A. bitorquis* mushroom farms was 115 phorids and 33 sciarids. The predominance of phorids over sciarid flies in this mushroom species crops was also registered.

The GLM developed to check the effect of the three factors ("species" "season" and "stage") as well as their interactions on the studied variables showed "species" and "stage" and the interaction between "species and season" to be statistically significant ($p<0.001$, F-test) on the incidence of phorid flies, but there were no significance for "season" nor its interaction with the remaining factors ($p> 0.05$, F-test) (Table 1). The multiple regression analysis (Table 2) showed all of the growing stages as statistically significant ($p<0.0001$, F-test), regardless of the species of *Agaricus*, the levels of phorids decreasing in the case of "incubation" stage and increasing for all the others stages. Of note is that *Agaricus bisporus* factor was also statistically significant ($p<0.0001$, F-test), the incidence of phorid flies decreasing, in general terms, on those farms.

Summarizing, growing "stage" was the main factor for the incidence of phorid flies in mushroom farms. The "species" of *Agaricus* also was influential, but the incidence of *M. halterata* increased on *A. bitorquis* farms, probably due to the longer

period of the growing stages in these crops, which allowed the appearance of the second generation of flies entirely developed inside the growing substrates.

[Table 2]

3.3 Study of the phoretic role of *M. halterata* as vector of *M. lambi*

The GLM developed to check the effect of the three factors ("species" "season" and "stage") as well as their interactions on the studied variables showed "species" and "stage" factors and the interaction between those factors as statistically significant ($p<0.001$, F-test) on the percentage of phorid vectors, meanwhile there was no significance in "season" factor and the rest of interactions between them (Table 1). The multiple regression analysis (Table 2) showed "incubation" and "casing" stages as statistically significant ($p<0.0001$, F-test), regardless of the species of *Agaricus*, the percentage of phorid vectors increasing in both stages, while "fourth flush" and "fifth flush" stages were also statistically significant, but only for *A. bisporus* crops in which the percentage of phorid vectors increased. That is, the percentage of phorid vectors was similar in both *Agaricus* crops during the incubation-induction periods (time considered as infection periods) and the three first flushes. However, at the end of the crop, this value had only increased in *A. bisporus* crops.

The GLM developed to check the effect of the three factors ("species" "season" and "stage") and of their interactions on the load of mites carried on each phorid vector showed "species" and "stage" and the interaction between them to be statistically significant ($p<0.001$, F-test), but there were no significance for "season" and the rest of the interactions (Table 1). The multiple regression analysis (Table 2) showed "*Agaricus*

bisporus" to be statistically significant ($p<0.0001$, F-test), increasing the load. On the other hand, the "incubation" stage was also statistically significant, increasing the load, but not in *A. bisporus* crops. That is to say that the number of mites carried on each phorid vector was higher almost throughout the growth cycle of *A. bisporus*, except during the incubation stage when the value was lower for *A. bisporus* that for *A. bitorquis* crops.

[Figure 1]

4 DISCUSSION

Clift & Toffolon (1981) demonstrated than the myceliophagous mite *Microdispus lambi* was capable of reproducing on *A. bisporus* as on *A. bitorquis* mycelium, although it could not sustain itself once the mycelium had completely colonised the compost[39]. Contrary to *Agaricus bisporus* mushroom crops[21], *M. lambi* appears not to be a pest on *A. bitorquis* mushroom farms in Spain. The incidence of myceliophagous mites in the growing substrates of summer mushrooms did not reach appreciable levels at any point during the crop cycle (Figure 1a). Both species of *Agaricus* show differences in their susceptibility to attack by pathogens, such as the fungi that cause diseases like " false truffle " and "dry bubble"[13,16,20]. As regards flies, the lower incidence of mushroom sciarids in summer mushroom crops compared to that observed in *A. bisporus* farms has been established[40], but it is not clear whether *A. bitorquis* is inherently less suitable as a host for *L. agarici* (synomysed with *L. auripila*) or whether a temperature effect is operating. The preference of *Megaselia halterata* for particular materials or species of mushroom has also been established[27-28,31-32], but to the best of our knowledge, there

have been no studies published on the incidence of this in *A. bitorquis* farms. It is possible that the absence of mites in *A. bitorquis* crops could be due to the low presence, in these farms, of the phorid flies, that are the main phoretic vectors for mites in *A. bisporus* farms[25].

The results of the present paper point to an important presence of phorid flies on *A. bitorquis* farms, normally higher than on *A. bisporus* crops (Figure 1b). This might be due to the longer period of the growing stages, which would allow the second generation of flies to completely develop inside the growing substrates. That is to say, *M. halterata* is obviously a pest of *A. bitorquis* mushroom crops. The percentage of phorids carrying mites registered during infection periods (incubation-induction stages) was not statistically different between both *Agaricus* crops (Figure 1b), while the number of phoretic mites (load) was significantly higher in the incubation stage of *A. bitorquis* compared to *A. bisporus* (Figure 1c). In other words, *A. bitorquis* growing substrates had the same infestation by *M. lambi* as *A. bisporus* growing substrates. But this mite did not install itself as a pest in summer mushroom crops. The threshold temperature of development (female) for *M. lambi* has been established at 9 ºC, and it is known that all life stages of the mite die when exposed to a constant temperature of 35 ºC for 24 h, or 32 ºC for 12 days[41]. However, the temperature registered inside the summer mushroom substrates did not reach these levels in either of the crops studied (data not shown). Whatever the case, the results of this study seem to contradict the possibility described in the literature[39] that *M. lambi* can reproduce on *A. bitorquis* species.

At the end of the crop cycle, the percentage of phorid vectors had only increased in *A. bisporus* crops, coinciding with the high incidence of mites in the substrates of this mushroom species. The phorids flies emerging from the summer mushroom crops did not carry mites on them due to the absence of mites in *A. bitorquis* mushroom substrates. After the cycle, new emerged flies would usually be attracted by the volatiles from the growing mycelium of new productive cycles. If those flies pick up mites they would probably infect nearby crops and contribute to the spread of *M. lambi* from infected crops to uninfected farms[25]. However, in *A. bitorquis* farms, the propagation of mites would be stopped. This difference between mushroom species, regarding the increment or reduction in the number of phorectic flies (in *A. bisporus* and *A. bitorquis* crops, respectively), explains the suitability of summer mushroom crops as a useful tool for the Integrated Pest Management (IPM) of *Microdispus lambi*, especially during outbreaks of mites in the production areas. However, it is necessary to continue the search for mechanisms to control the phorid fly to reduce the damage it causes and the consequences of its action as vector of other pests and diseases.

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Table 1. Statistics of goodness of fit for the full model obtained by GLM for the response and predictive (factors) variables

Variable†	R^2	Factor	Sum of squares	d.f.	F	p-value
Presence of mites (mites/120 g)	75.70	Full model	541.95	29	6.44	0.0000
		Species	74.71	1	25.76	0.0000
		Season	1.00	2	0.17	0.8416
		Stage	209.37	4	18.05	0.0000
		Species*Season	15.03	2	2.59	0.0832
		Species*Stage	87.86	4	7.57	0.0001
		Season*Stage	11.57	8	0.50	0.8523
		Species*Season*Stage	15.17	8	0.65	0.7293
		Residual	173.99	60		
Incidence of phorids (adults/day)	69.07	Full model	317.48	47	4.47	0.0000
		Species	51.67	1	34.15	0.0000
		Season	1.62	2	0.53	0.5877
		Stage	180.39	7	17.03	0.0000
		Species*Season	25.58	2	8.46	0.0004
		Species*Stage	17.35	7	1.64	0.1342
		Season*Stage	7.85	14	0.37	0.9800
		Species*Season*Stage	7.69	14	0.36	0.9819
		Residual	142.20	94		
Phorids as Vectors (%)	55.58	Full model	523.73	47	4.00	0.0000
		Species	131.06	1	47.04	0.0000
		Season	3.06	2	0.55	0.5793
		Stage	143.59	7	7.36	0.0000
		Species*Season	18.86	2	3.39	0.0381
		Species*Stage	83.21	7	4.27	0.0004
		Season*Stage	47.58	14	1.22	0.2748
		Species*Season*Stage	26.43	14	0.68	0.7905
		Residual	256.32	92		
Load (mites/vector)	51.77	Full model	30.52	47	2.12	0.0010
		Species	6.69	1	21.89	0.0000
		Season	1.04	2	1.69	0.1894
		Stage	8.60	7	4.02	0.0007
		Species*Season	1.29	2	2.11	0.1271
		Species*Stage	5.02	7	2.34	0.0300
		Season*Stage	5.91	14	1.38	0.1783

Species*Season*Stage	3.96	14	0.92	0.5359
Residual	28.44	93		

[†]A natural logarithmic transformation of data concerning the presence of mites and incidence of phorids was used. An SQRT transformation of data concerning the percentage of phorids as vectors and load was used.

Table 2. Regression coefficients for the predictor factors (k-1 dummy variables, being k the nº of levels for each factor) of the presence of mites, incidence of phorids, percentage of phorids as vectors and load of mites on each phorid vector and statistics for goodness of fit*

		Presence of mites (mites/120g)	Incidence of phorids (adults/day)	Phorids as vectors (%)	Load (mites/vector)
Factors	Dummy variables	Coefficients†			
	Constant	1.31	202.23	5.72	0.70
Species	*Agaricus bisporus*		-282.52		1.43
Growth stage	Incubation		-22.39	15.29	2.99
	Casing			15.07	
	First flush		153.94		
	Second flush		286.44		
	Third flush	27.64	324.64		
	Fourth flush		346.79		
	Fifth flush		172.45		
Interaction	(*A. bisporus*)*(Incubation)				-2.99
	(*A. bisporus*)*(First flush)	54.97			
	(*A. bisporus*)*(Third flush)	477.45			
	(*A. bisporus*)*(Fourth flush)			24.75	
	(*A. bisporus*)*(Fifth flush)	3908.43		31.42	
	(*A. bisporus*)*(Spring)		76.58		
n		90	142‡	140‡	141‡
F		47.95	23.11	21.70	13.52
P		<0.001	<0.001	<0.001	<0.001
R^2		69.29	58.16	39.14	22.84
SEE		1.61	1.20	1.88	0.58

*All coefficients shown are significant at $P < 0.05$. Empty cells means that the coefficients are not significant ($P > 0.05$).

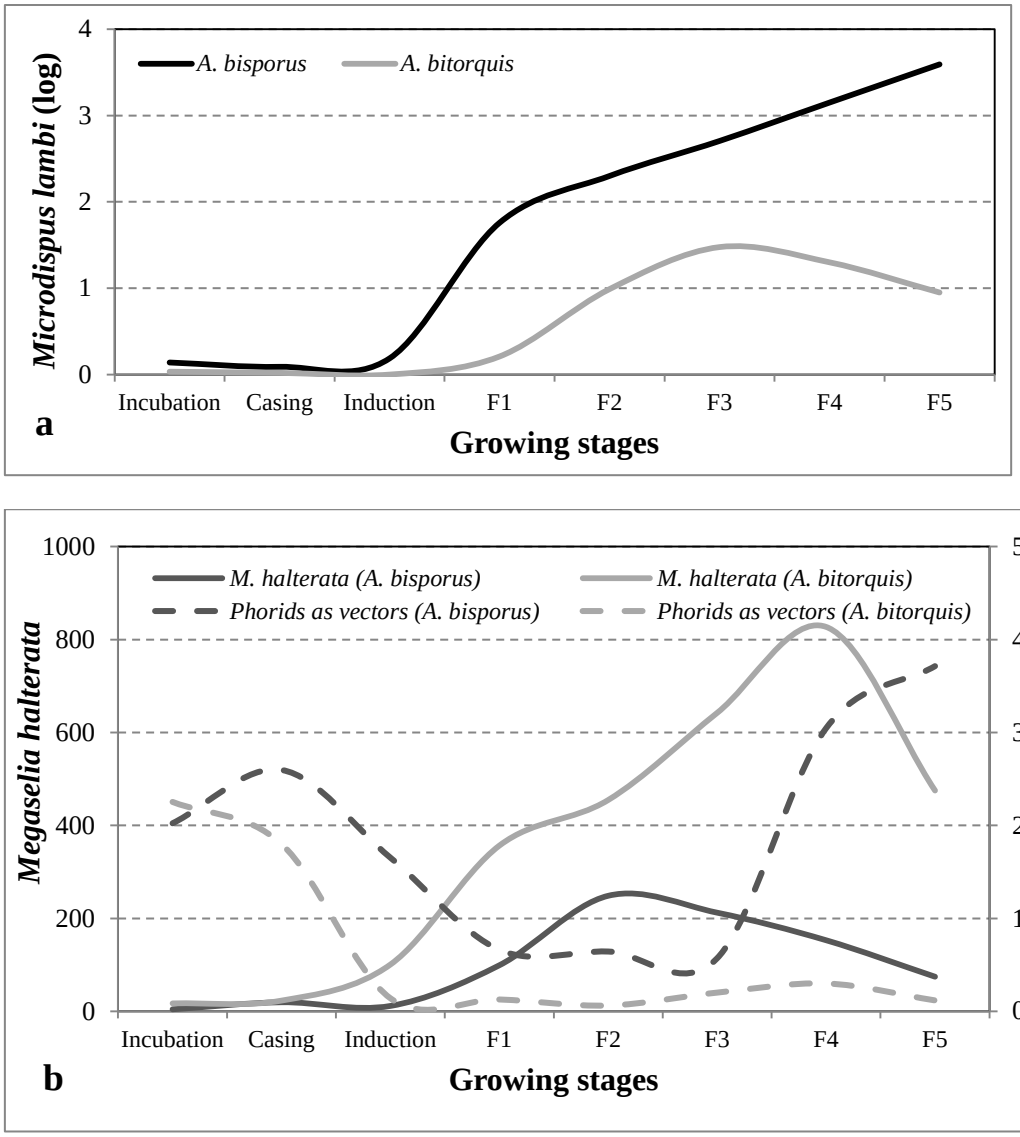
[†]The response variables were not LOG or SQRT transformed.

[‡]There were between 2 to 4 missing data for these interest variables

Figure legends

Figure 1. a) Progression of the presence of *M. lambi* (mites/120 g of substrate sample) in *Agaricus bisporus* and *A. bitorquis* crops. b) Progression of the incidence of *M. halterata* (total adults captured per day) and of phorids as vectors of *M. lambi* mites (%) in the different periods of the growth cycle in both mushroom species. c) Progression of the load (number of mites carried by each phorid vector) in the different periods of the growth cycle in both mushroom species.

[†]F1: first flush; F2: second flush; F3: third flush; F4: fourth flush; F5: fifth flush



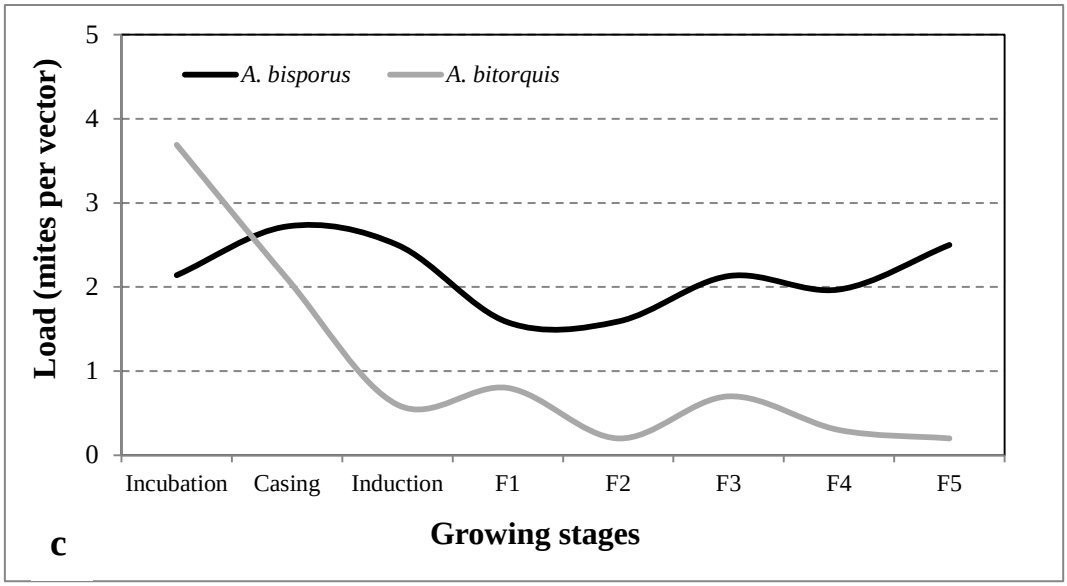


Figure 1